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Mini-review

Microbial responses to a changing environment: implications for the future functioning of terrestrial ecosystems

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ABSTRACT

In this review, we present a conceptual model which links plant communities and saprotrophic microbial communities through the reciprocal exchange of growth-limiting resources. We discuss the numerous ways human-induced environmental change has directly and indirectly impacted this relationship, and review microbial responses that have occurred to date. We argue that compositional shifts in saprotrophic microbial communities underlie functional responses to environmental change that have ecosystem-level implications. Drawing on a long-term, large-scale, field experiment, we illustrate how and why chronic atmospheric N deposition can alter saprotrophic communities in the soil of a wide-spread sugar maple (*Acer saccharum*) ecosystem in northeastern North America, resulting in the slowing of plant litter decay, the rapid accumulation of soil organic matter, and the accelerated production and loss of dissolved organic carbon (DOC). Compositional shifts in soil microbial communities, mediated by ecological interactions among soil saprotrophs, appear to lie at the biogeochemical heart of ecosystem response to environmental change.

Introduction

Soil harbours a phylogenetically diverse community of saprotrophic microorganisms whose physiological activities mediate the biogeochemical cycling of carbon (C) and nitrogen (N) at local, regional and global scales. These communities are structured by the physical environment as well as the availability of growth-limiting resources entering soil (i.e., organic

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compounds in plant detritus). Presently, human activity is affecting both the physical conditions and the availability of resources to soil microbial communities at a global scale, but the implications for the future functioning of terrestrial ecosystems are unclear. In this review, we discuss the ways in which humans are altering the ecological constraints on saprotrophic microbial communities in soil, the compositional and functional responses that may result, and identify knowledge gaps that limit our ability to anticipate the response of microbial communities and the ecosystem processes they mediate. Our review centers on microbial processes that govern the cycling and storage of C and N in soil; specifically, we focus on saprotrophic fungal communities, their interaction with other saprotrophs (i.e., *Actinobacteria*) during plant litter decay, and how increased atmospheric N deposition may alter the composition and function of saprotrophic microbial communities in soil.

Plant communities and saprotrophic microbial communities are linked through the reciprocal exchange of growth-limiting resources (solid lines in Fig 1), a relationship that human-induced environmental change will continue to alter into the future (dotted lines in Fig 1). Plant productivity in a wide range of habitats is governed by the amount of inorganic N liberated during the microbial decay of plant detritus (Vitousek & Howarth 1991). Although saprotrophic bacteria and fungi are primary agents of decay, the supply of N to plants can be supplemented by the mycorrhizal mobilization of N from soil humus (Talbot *et al.* 2008), especially by ectomycorrhizal fungi in boreal forests and northern heathlands (Read & Peres-Moreno 2003; Lindahl *et al.* 2007; not depicted in Fig 1). In turn, saprotrophic microbial growth in soil is constrained by the amount of energy that can be enzymatically harvested from plant detritus shed above- and belowground. Because of this relationship, human-induced environmental change that modifies the production and biochemical composition of plant detritus (dotted lines in Fig 1) also alters the supply of limiting resources to saprotrophic soil microorganisms, which shape their composition, the rate at which they release N from plant detritus, as well as the rate at which they form soil organic matter (Fig 1). Because soil organic matter contains twice as much C as the Earth's atmosphere, the response of soil microbial communities to environmental change has global consequences for the cycling and storage of C in terrestrial ecosystems.

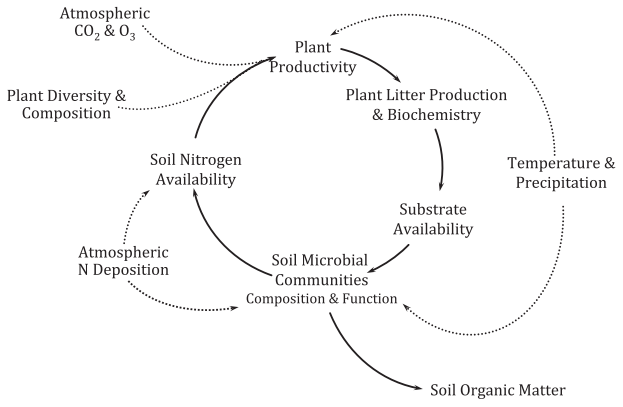

Fig 1 – Plant and saprotrophic microbial communities in soil are linked *via* the reciprocal exchange of growth-limiting resources. Saprotrophic microbial communities are structured by the availability of organic substrates in plant detritus that can be used for energy; they also are structured by the physical environment. In turn, plant communities are broadly limited in their growth by the amount of N made available during the microbial decay of dead plant tissues. These relationships are illustrated with the solid arrows. In some ecosystems, the amount of N plants obtain is supplemented by ectomycorrhizal fungi, which mobilize organic N (not depicted here). A wide array of global change factors have the potential to alter microbial communities directly or indirectly *via* their influence on plant communities.

Over the past decade, our knowledge of the multiple ways human-induced environmental change can influence soil microbial communities and biogeochemical cycles has rapidly advanced, although it is far from being complete. For example, we do not yet understand whether changes in microbial processes that mediate biogeochemical cycles in soil occur through a shift in community composition (i.e., community membership and species abundance), a shift in the organisms actively mediating a biogeochemical process (i.e., which species are active, with no change in community membership), or through some combination of both. This critical gap in our knowledge extends to most facets of soil microbial ecology, and it remains an important scientific challenge.

Despite this limitation, we have learned that changes in plant litter production in response to CO_2 and O_3 accumulating in the atmosphere, reductions in plant diversity, increasing soil temperatures, altered precipitation regimes, and greater rates of atmospheric N deposition can exert direct (i.e., alter physiological activity) and indirect (i.e., alters resource supply, which, in turn, alters physiological activity) influences on soil microbial communities, thereby modifying the biogeochemistry of C and N in soil. We also have learned that many of these responses can be divergent. For example, greater detrital production under elevated atmospheric CO_2 can increase, decrease, or have no effect on rates of soil N cycling and the subsequent supply of soil N to plants (de Graff *et al.* 2006; Reich *et al.* 2006); rates of soil N cycling beneath plants exposed to elevated O_3 can slow (Holmes *et al.* 2003) and remain unchanged (Kanerva *et al.* 2006). Likewise, higher levels of plant diversity can increase fungal abundance and rates of soil N cycling (Zak *et al.* 2003) as well as have no effect whatsoever on microbial community composition and biogeochemical processes (Wardle *et al.* 1999). Although some microorganisms may physiologically acclimate to warmer soil temperatures by lowering respiration rates (Luo *et al.* 2001; Bradford *et al.* 2008), others do not (Kirchbaum 2006; Malcolm *et al.* 2009; Vanhala *et al.* 2010). Notwithstanding these divergent responses, one consistent observation has emerged: the presence of particular plant species and their abundance in plant communities can shape both the composition and function of microbial communities in soil (Kourtev *et al.* 2002; Edwards & Zak 2010; Eisenhauer *et al.* 2010). This observation provides further support for the reciprocal link between plant and saprotroph communities depicted by the solid lines in Fig 1.

There is a critical need to uncover the mechanism(s) underlying the disparate observations described above, because they generate substantial uncertainty for our ability to predict the magnitude and direction by which the multiple facets of environmental change (dotted lines in Fig 1) will alter the future functioning of terrestrial ecosystems. We argue that conflicting microbial responses to environmental change arise from compositional shifts in microbial communities that have functional implications for the biochemical cycling of C and N in soil, an idea supported by accumulating evidence (Yuste *et al.* 2010). At a fundamental level, it is the expression of fungal and bacterial genes encoding enzymes that catalyze biochemical reactions, which, in turn, mediate biogeochemical processes in soil. Moreover, the presence of these expressed genes and their pre- and post-translational regulation influences competitive interactions among organisms, which shape community composition, as well as modulate the decay rate of plant detritus and the subsequent formation of soil organic matter. Because soil saprotrophs dramatically differ in their physiological capacity to metabolize the biochemical components of plant detritus (Goodfellow & Williams 1983; Baldrian 2006; Steffen *et al.* 2007), a change in community membership should have functional consequences for the biogeochemical cycling of C and N in soil. Nonetheless, many challenges remain before we are able to connect molecular processes which operate on the time step of seconds to minutes (i.e., gene transcription and translation) to competitive interactions that structure microbial communities, and, ultimately, to the decadal response of biogeochemical processes to environmental change (i.e., a change in soil C storage).

To support our argument and illustrate these points, we drawn on a long-term, large-scale field experiment aimed at understanding ecosystem response to future rates of atmospheric N deposition. We focus on key biochemical processes regulated by soil fungi and *Actinobacteria* during the decay of plant detritus, and how compositional changes in saprotrophic communities can have functional implications for ecosystem response to environmental change. It is beyond the scope of this paper to review the myriad of microbial responses to the numerous facets of environmental change; rather, we center the remaining portion of our review on the direct and indirect ways future rates of atmospheric N deposition will alter saprotrophic fungal and actinobacterial communities in a temperate forest soil, the process of plant litter decay, and soil C storage. Here, we provide the background and develop the rationale for why chronic atmospheric N deposition may alter the composition and function of saprotrophic fungal and actinobacterial communities in soil. We then present experimental evidence supporting our ideas, and we conclude by demonstrating how greater insight into biogeochemical processes can be gained by understanding links between composition and function in soil microbial communities.

Atmospheric N deposition, soil microbial communities and ecosystem response

Over the past 150 yr, human activity has dramatically altered the global N cycle by increasing the amount of biologically available N in the biosphere (Canfield *et al.* 2010). For example, atmospheric nitrogen (N) deposition has increased an order of magnitude across large areas of the Earth, especially in the Northern Hemisphere (e.g., from 50–100 to 1 500–2 000 mg N m^{-2} yr^{-1}; Galloway *et al.* 2004); this trend is anticipated to extend well into the next century. The stimulation of net primary productivity (NPP) as N availability increases in N-limited northern forest has been considered the primary mechanism contributing to the globally important C sink residing in these ecosystems (Townsend *et al.* 1996; Nadelhoffer *et al.* 1999; Currie *et al.* 2004). In Fig 1, this response is illustrated by the dashed line from atmospheric N deposition to soil N availability and the clockwise solid arrow pointing to plant productivity. Biogeochemical models centered on this assumption projected that atmospheric N deposition might account for an additional 0.1–2.3 Pg of annual C storage in the Northern Hemisphere (Schindler & Bayley 1993, Townsend *et al.* 1996, Holland *et al.* 1997), but there remains substantial disagreement over the magnitude of the effect (Nadelhoffer *et al.* 1999, Magnani *et al.* 2007, deVries *et al.* 2008, Reay *et al.* 2008). This uncertainty could plausibly arise from additional mechanisms of ecosystem C storage that have not been previously considered.

There is a microbial mechanism by which atmospheric N deposition could increase C storage in terrestrial ecosystems that remains largely unexplored, and it is not a component of biogeochemical models estimating ecosystem C storage on an N-enriched Earth. Under laboratory conditions, the synthesis of lignolytic enzymes by some basidiomycetes can be repressed by high levels of inorganic N (e.g., *Phanerochaetes* Boominathan *et al.* 1990; Vanderwoude *et al.* 1993; Worrall *et al.* 1997). The molecular mechanism mediating a decline in lignolytic activity under laboratory conditions is the transcriptional down regulation of fungal genes encoding phenol oxidase, Mn-peroxidase and lignin peroxidase (Tien & Tu 1987; Brown *et al.* 1991; Li *et al.* 1994). Although this response is not universal (Worrall *et al.* 1997; Sun *et al.* 2004), a decline in the transcription of lignocellulolytic genes under field conditions could translate to the slowing of litter decay and an accumulation of soil organic matter (Carreiro *et al.* 2000). In Fig 1, this direct effect is illustrated by the dotted line connecting atmospheric N deposition to microbial community composition and function; it substantively differs from the indirect effect of atmospheric N deposition mediated through change in plant litter production and biochemistry, as described above. If higher levels of inorganic N in the environment suppress lignin decomposition and thus limit access to other usable energy sources (cellulose, hemicellulose) by these organisms, then it could reduce their competitive ability in the soil environment, plausibly opening a niche for other organisms with lignocellulolytic capacities to occupy; such a response could alter the community of microorganisms actively mediating lignocellulose decomposition.

Filamentous *Actinobacteria* (e.g., *Streptomyces* spp.) are known to decompose lignocellulose, and their importance during the process of litter decay may be increased by chronic N deposition. Although these organisms cannot completely metabolize lignin to CO_2, they produce soluble polyphenolics as end products of lignocellulolytic metabolism (Godden *et al.* 1992; Berrocal *et al.* 1997). More importantly, the rate of

lignocellulose metabolism by some species of *Actinobacteria* can be stimulated by high N availability (Barder & Crawford 1981; Giroux *et al.* 1988), presenting the possibility that lignocellulolytic *Actinobacteria* may become more important agents of decay as human activity continues to increase N availability in the environment. If chronic N deposition causes *Actinobacteria* to become more active agents of lignocellulose metabolism, then such a shift in saprotrophic community composition should have biogeochemical consequences (e.g., greater production of dissolved organic C, less lignin being metabolized to CO_2) that are observable under field conditions.

Additionally, the later stages of litter decay, which are dominated by basidiomycete decomposition of lignin (Frankland 1998), are also slowed in detritus with a high initial N concentration (Berg & Matzner 1997; Berg & Meentemeyer 2002), despite an initial acceleration of decay. If atmospheric N deposition chronically increases leaf litter N and elevates inorganic N in soil solution, then it could potentially: (i) repress lignocellulolytic activity by fungal saprotrophs; (ii) open a niche for lignocellulolytic *Actinobacteria* to occupy, subsequently altering saprotrophic community composition; and (iii) slow litter decay, increase dissolved organic C production, and foster organic matter accumulation. Recent meta-analyses provide evidence that declines in microbial activity, the slowing of decay, and the surface accumulation of organic matter are common responses to anthropogenic N deposition in a wide range of terrestrial ecosystems (Liu & Greaver 2010). Although not all terrestrial ecosystems respond in such a manner (Hobbie 2008; Papanikolaou *et al.* 2010), this observation suggests a common set of microbial responses may underlie ecosystem response to anthropogenic N deposition.

Microbial and ecosystem responses to atmospheric N deposition: results of a long-term, large-scale field experiment

Over the past 17 yr, we have experimentally simulated future rates of atmospheric NO_3^- deposition in replicate stands of a northern hardwood forest ecosystem stretching across a 500 km climatic gradient (Fig 2). Our study sites deliberately encompass the north–south geographic range of sugar maple-dominated (*Acer saccharum*) northern hardwood forests in the Great Lakes region of North America (Braun 1950), enabling us to generalize our experimental results across this geographic region. These sites are floristically and edaphically matched (>80 % sugar maple on Typic Haplorthods; see Burton *et al.* 1991), but they differ in climate along the north–south latitudinal gradient (Fig 2). The study sites also span an atmospheric N deposition gradient, over which NO_3^-–N composes ~60 % of wet-plus-dry deposition (see Pregitzer *et al.* 2004 for details). There are six 30 m × 30 m plots at each study site. Three plots at each site receive ambient atmospheric N deposition. The other three plots at each site receive ambient N deposition plus 3 g NO_3^-–N m^{-2} yr^{-1}, a rate approaching that expected by 2050 across large portions of North America and other regions of the Earth (Galloway *et al.* 2004). The additional NO_3^- is delivered over the growing season in six equal applications of solid $NaNO_3$ pellets, which

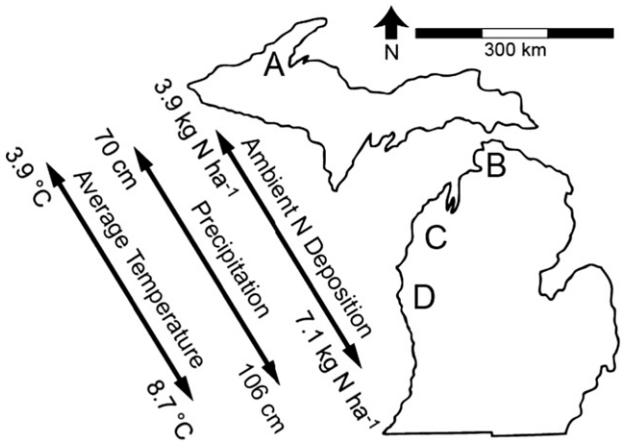

Fig 2 – Distribution of replicate study sites spanning the north-south range of northern hardwood forests in the Upper Great Lakes region of eastern North Amercia.

are broadcast over the forest floor. To date, we have manipulated N deposition for 17 continuous years across the entire geographic range of this ecosystem. To the best of our knowledge, this is the largest and longest running replicated manipulation of atmospheric N deposition in any forest ecosystem.

Has chronic N deposition slowed litter decay?

For chronic N deposition to slow decay *via* the mechanisms we have described above, it first must elevate inorganic N in soil solution as well as increase the N concentration of plant detritus. Since 1995, simulated N deposition has consistently increased soil NO_3^- concentrations, resulting in significant leaching losses of both inorganic and organic N (Pregitzer *et al.* 2004), which is now equivalent to ~70–90 % of the N added in our simulated N deposition treatment. At present, inorganic N concentrations in soil solution are significantly greater under simulated N deposition (2.3 *vs.* 8.9 μg NO_3^-–N g^{-1}, $P \leq 0.001$; ambient *vs.* simulated N deposition; Zak *et al.* 2009), which has undoubtedly produced the greater N concentration of canopy leaves (19.2 *vs.* 22.8 mg N g^{-1}, $P \leq 0.001$; Zak *et al.* 2009) as well as leaf litter (8.1 *vs.* 10.1 mg N g^{-1}, $P \leq 0.001$; Pregitzer *et al.* 2008); simulated N deposition has not altered the lignin concentration of leaf litter (146 *vs.* 141 mg g^{-1}, $P = 0.656$; Eikenberry 2004). Simulated N deposition has not changed fine root N concentration (17.5 *vs.* 19.1 mg N g^{-1}, $P = 0.342$; Eikenberry 2004), lignin concentration (376 *vs.* 366 mg g^{-1}, $P = 0.101$) or turnover (0.62 yr^{-1} *vs.* 0.57 yr^{-1}; $P > 0.10$; Burton *et al.* 2004). Because the production of leaf and fine root detritus does not differ between ambient and simulated N deposition (Burton *et al.* 2004), the overall effect of simulated N deposition on above- and belowground litter has been to increase leaf litter N concentration alone. Taken together, these observations indicate that the availability of organic substrates to soil saprotrophs has not been altered by chronic N deposition (solid lines; Fig 1), presenting the possibility that chronic N deposition could have a direct (i.e., high NO_3^- concentration) and indirect (i.e., high leaf litter N)

negative effect on the saprotrophic organisms mediating plant litter decay (dotted line; Fig 1).

Several complementary lines of evidence are consistent with the idea that higher inorganic N concentrations in soil solution and N-rich leaf litter have decreased lignolytic activity *via* a direct effect on saprotrophic organisms (i.e., down regulating the synthesis of lignolytic extracellular enzymes). Fungal class II peroxidases (i.e. lignin peroxidase, manganese peroxidase and versatile peroxidase) as well as phenol oxidases of the laccase-type are the main extracellular enzymes that oxidatively attack lignin as well as a wide range of polyphenols, including humic compounds in soil organic matter (Ten Have & Teunissen 2001; Baldrian 2006; Hofrichter *et al.* 2010). Furthermore, there are indications that dye-decolorizing peroxidases and heme-thiolate peroxidases can act on lignin structures (Hofrichter *et al.* 2010). These enzymes are produced in large quantities by soil fungi (Kirk & Farrell 1987; Worrall *et al.* 1997; Hibbett & Donoghue 2001), but are produced in much lower amounts by lignocellulolytic *Actinobacteria* as well as differing in their relative amount (Ramachandra *et al.* 1987). If the transcription of basidiomycete genes encoding these enzymes is decreased by high inorganic N (Tien & Tu 1987; Brown *et al.* 1991; Li *et al.* 1994), or if *Actinobacteria* become more important during lignin decay, these responses have the potential to decrease the synthesis of extracellular enzymes as well as the subsequent oxidation of lignin, humus and other polyphenols. In forest floor (Oe/Oa soil horizons), simulated N deposition has reduced the activity of phenol oxidase and peroxidase to a substantial degree (~−33 %; Fig 3, upper panel); this effect was not apparent in surface mineral soil (Fig 3; lower panel). These observations are consistent with the expectation that high inorganic N concentrations, induced by simulated N deposition, can inhibit lignolytic activity. This effect also could plausibly arise from the high N concentration in leaf litter (Berg & Matzner 1997; Berg & Meentemeyer 2002). At this time, we are unable to discern whether higher inorganic N concentrations in soil solution, leaf litter, or both have decreased phenol oxidase and peroxidase activity.

To determine whether reductions in lignolytic enzyme activity have slowed litter decay, we inventoried organic matter residing in plant and soil pools in all four study sites (Pregitzer *et al.* 2008; Zak *et al.* 2009). Simulated N deposition significantly increased organic matter stored in forest floor and mineral soil (0–70 cm; Pregitzer et al. 2008; Zak *et al.* 2009). However, organic matter primarily accumulated in forest floor (Oa/Oe, 803 *vs.* 1212 g Cm^{-2}; $P = 0.009$) and surface mineral soil (0–10 cm, 1753 *vs.* 2070 g Cm^{-2}; $P = 0.072$). Because aboveground litter production was equivalent under ambient and simulated N deposition, organic matter has accumulated due to the slowing of plant litter decay (Zak *et al.* 2009). Further evidence for slower decomposition is the significantly longer turnover of forest floor under simulated N deposition (4.3 *vs.* 6.8 yrs; $P = 0.047$; Zak *et al.* 2009), as well as the corresponding declines in lignolytic activity (Fig 3) and soil respiration (−15 %, $P \leq 0.001$; Burton *et al.* 2004).

Since 1996, dissolved organic C (DOC) in soil solution has been significantly greater under simulated N deposition (volume weighted mean concentration = 6.0 *vs.* 18.0 mg l^{-1}, $P \leq 0.001$), a response that has sustained a nearly two-fold

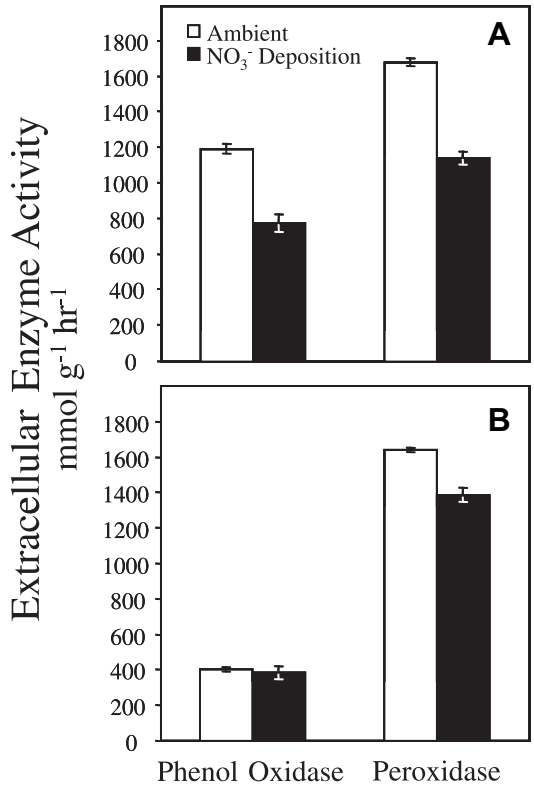

Fig 3 – Mean activity of lignolytic extracellular enzymes in forest floor (A) and surface soil (B) in the ambient and simulated N deposition. Data have been summarized from DeForest *et al.* (2004, 2005).

increase in DOC leaching (Pregitzer *et al.* 2004). Through ^{14}C dating, we have learned that DOC in both treatments is <50 yr old, and laboratory experiments confirm that the forest floor is the primary source of DOC (Smemo *et al.* 2006, 2007). Moreover, the concentration of soluble phenolics in DOC has increased by 50 % under simulated N deposition (Smemo *et al.* 2007). These changes in DOC export from soil did not result from an experimentally induced increase in soil pH (*sensu* Evans *et al.* 2008; pH (0–10 cm) = 4.58 *vs.* 4.70; $P = 0.149$), nor is there any relationship between soil solution pH and DOC production in our experiment ($P = 0.208$) as others have observed (Evans *et al.* 2008). Slower forest floor decomposition and the greater production of phenolic DOC are indications that simulated N deposition has altered the manner in which saprotrophic soil microorganisms are metabolizing plant litter into soil organic matter. From what we know of the lignocellulose metabolism by *Streptomyces* spp. (Barder & Crawford 1981; Mason *et al.* 1988; Vidal *et al.* 1989), the decline in decay and greater phenolic DOC production we have documented are consistent with the idea that actinobacterial lignocellulose decay has become more dominant under simulated N deposition. These biogeochemical responses also are consistent with the idea that high inorganic N in soil solution, as well as N-rich leaf litter, can repress lignolytic activity by litter-decaying fungi. If composition and function are linked in saprotrophic communities, then simulated N deposition should also alter the community of organisms mediating

plant litter decay as well as the manner by which they metabolize plant litter into soil organic matter and DOC.

Has chronic N deposition altered fungal and actinobacterial communities in soil?

We have endeavored to understand the composition of fungal and actinobacterial communities in northern deciduous forests (Edwards & Zak 2010, 2011; Eisenlord & Zak 2010) and their response to environmental change. Within the forest floor, the overwhelmingly saprotrophic basidiomycete community is dominated by Agaricomycetes, especially Agaricales (Edwards & Zak 2010). *Mycena* spp., *Marasmius* spp., *Collybia* spp, *Psathyrella* spp. and *Clitopilus* spp. are well represented in forest floor. Further, ascomycete diversity can be high in forest floor and dominated by *Pezizomycotina* (Edwards & Zak 2011). Although the ecology of most ascomycete spp. can only be surmised, cellobiohydrolase genes of ascomycete origin also dominate the forest floor (Edwards *et al.* 2008). In contrast, the mineral soil community has a higher proportion of ectomycorrhizal species (*Russula*, *Ramaria*, *Hebloma*, *Phyllopros, Paxillus and Sebacina* spp.), despite the fact sugar maple dominates the overstory and it associates with AM fungi (i.e. *Glomus*); saprotrophic *Gymnopus* spp. and *Clavulinopsis* spp. are present in high proportion (Edwards & Zak 2010). The community of forest floor *Actinobacteria* can be dominated by *Micromonosporaceae, Frankineae* and *Microbacteriaceae,* whereas mineral soil hosts a diverse assemblage of *Acidimicrobiales, Mycobacteriaceae, Streptosporangiaceae* and *Frankineae* (Eisenlord & Zak 2010).

We have argued that greater soil N availability could differentially modify competitive interactions between litter-decaying fungi and *Actinobacteria,* resulting in compositional shifts that elicit functional responses (i.e., slowing of decay and greater DOC production). Although basidiomycete laccase genes can be amplified from soil to assess their abundance (Luis *et al.* 2004; Kellner *et al.* 2009), there is a paucity of information on actinobacterial phenol oxidase, peroxidase and glycoside hydrolase genes (Kirby 2006), causing us to rely on a broader assessment of actinobacterial abundance (i.e., 16S rRNA). Contrary to our expectation, simulated N deposition has not significantly altered the abundance of basidiomycete laccase genes in the forest floor, nor has it altered the abundance of actinobacterial 16S rRNA genes (Fig 4). In part, these results arise from substantial site-to-site variability in gene copy number (see Hassett *et al.* 2009; Eisenlord & Zak 2010). Regardless of this variation, copies of actinobacterial 16S rRNA genes in mineral soil were lower under simulated N deposition ($P = 0.025$; Eisenlord & Zak 2010); basidiomycete laccase gene copies in mineral soil were not influenced by simulated N deposition (Hassett *et al.* 2009). Given these results, the biogeochemical responses to simulated N deposition that we have documented cannot be explained by a change in the abundance of these soil saprotrophs and thereby present several alternatives: the slowing of decay and increase in DOC production may result from changes in community composition, in the organisms which are actively metabolizing lignocellulose (i.e., those transcribing genes encoding lignocellulolytic enzymes), or through some combination of both.

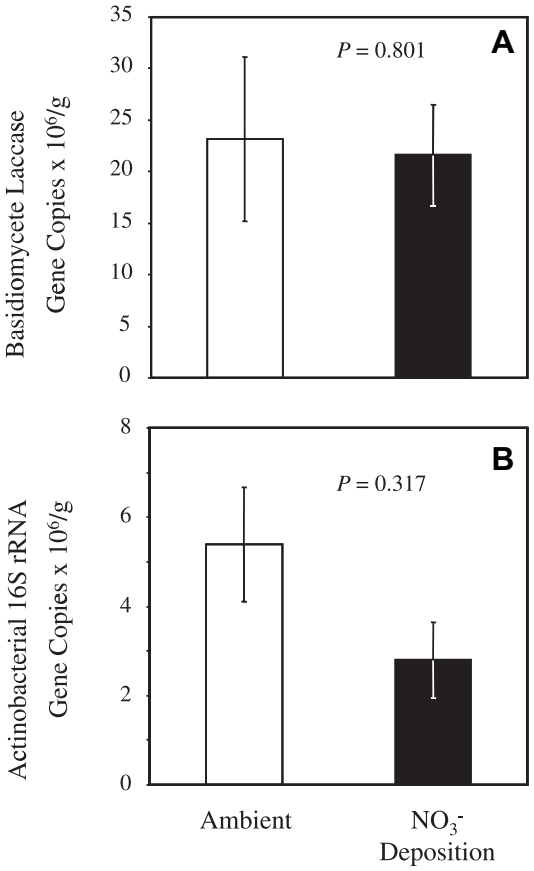

Fig 4 – Abundance of basidiomycete laccase genes (A) and actinobacterial 16S rRNA genes (B) in forest floor of ambient and simulated N deposition treatments. Data have been summarized from Hassett *et al.* (2009) and Eisenlord & Zak (2010).

To test these alternatives, we amplified portions of actinobacterial and fungal rRNA genes to gain insight into community composition, which we analyzed using terminal fragment restriction polymorphisms. These analyses revealed that the composition of *Actinobacteria*, as well as fungal community composition, was significantly altered by simulated N deposition (Fig 5). Further cloning and sequencing of actinobacterial 16S rRNA demonstrated that simulated N deposition elicited a significant membership change in both forest floor and surface soil communities, as well as significant differences in the phylogenetic diversity of forest floor *Actinobacteria* (Eisenlord & Zak 2010). For example, members of the *Acidimicrobiales* responded positively to simulated N deposition, whereas members of the *Streptosporangiaceae* responded negatively. We do not know how robust this change is over time, or how it corresponds with the observed change in fungal community composition. Surprisingly, the lignin-decomposing family *Streptomycetaceae*, which is the most widely studied *Actinobacteria* family, accounted for only 4 % of the forest floor community and <1 % of the surface soil community, suggesting these organisms might not dominate the process of lignin metabolism in our experiment.

Compositional shifts in the actinobacterial community occurred in concert with the slowing of decomposition and

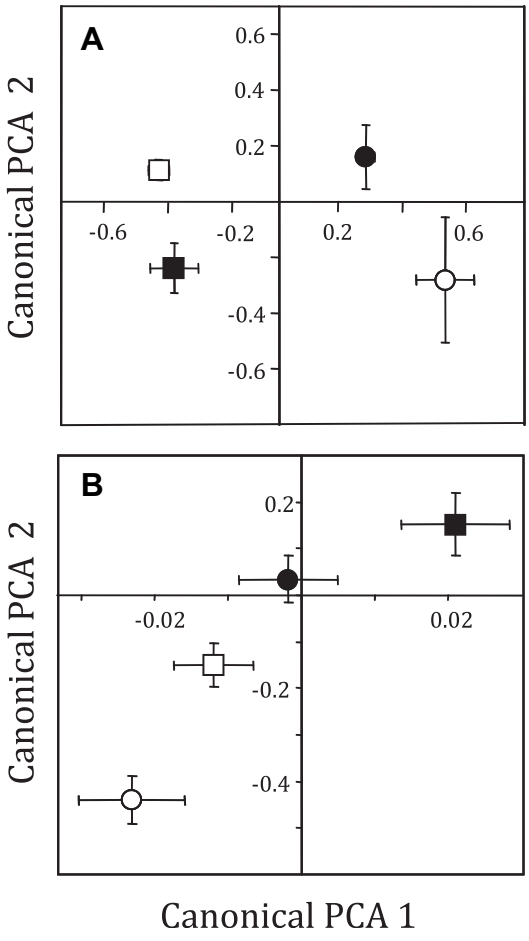

Fig 5 – Actinobacterial (A) and fungal (B) community composition is significantly altered by simulated N deposition ($P \leq 0.01$). Depicted are means for ambient (open) and simulated N deposition (shaded) in forest floor (squares) and surface soil (circles) across all four sites. Actinobacterial 16S rRNA genes were amplified using 338F/Act1159R; amplicons were digested with Taq1 & DpnII. Fungal ITS/28S rRNA genes were amplified using ITS1F/TW-13, and PCR products were digested with HAE III. We conducted redundancy analyses of the resulting actinobacterial and fungal T-RFLPs.

soil respiration, declines in lignolytic activity and increases in phenolic DOC production. Moreover, they support the idea that a shift in microbial community composition underlies the biogeochemical responses elicited by simulated N deposition. At present, we are unable to establish a causal link between changes in fungal and actinobacterial communities under simulated N deposition and the slowing of plant litter decay. Much is left to learn regarding the identity of the fungi and *Actinobacteria* that have responded positively and negatively to simulated N deposition, as well as why changes in microbial community composition have altered the cycling and storage of C in soil. Regardless of these limitations, our results suggest that compositional shifts in soil microbial communities can elicit functional responses that influence the biogeochemical cycling of C in soil.

Has chronic N deposition altered the transcription of fungal genes with lignocellulolytic function?

Changes in fungal and actinobacterial community composition described above present the possibility that the community of organisms actively metabolizing plant detritus into soil organic matter has been altered by simulated N deposition. To address this alternative, we identified transcriptionally expressed fungal genes encoding lignocellulolytic enzymes as well as others in Site D (Fig 1; Kellner *et al.* 2010). By isolating total RNA from the forest floor and reverse transcription, we synthesized cDNA, which provided templates for subsequent detection of fungal transcripts (Kellner *et al.* 2010). Manganese peroxidase, laccase and cellobiose dehydrogenase are key enzymes regulating lignocellulose metabolism, and we found no evidence that simulated N deposition circumvented their expression by basidiomycete and ascomycete fungi (Kellner *et al.* 2010). We also found no evidence that simulated N deposition influenced the transcription (presence) of basidiomycete and ascomycete glycoside hydrolases mediating cellulose and hemicellulose depolymerization. However, only 22 of the 234 total detected transcripts occurred in both treatments, with the remainder uniquely occurring under ambient or simulated N deposition. Although the gene analysis was not exhaustive, this observation supports the idea that the identity of fungi actively metabolizing plant detritus into soil organic matter has been altered by simulated N deposition.

Due to limited knowledge of actinobacterial genes encoding lignocellulolytic enzymes (Kirby 2006), as well as the challenges involved in isolating actinobacterial transcripts, we presently are unable to gain equivalent insight into the community of *Actinobacteria* mediating lignocellulose decay in our experiment; this remains an important challenge in the context of our experiment as well as understanding the ecological functions of these organisms in soil. Our observations suggest that understanding the environmental regulation of fungal gene expression may underlie why simulated N deposition has decreased decay and altered soil C cycling. Although a wide array of fungal genes encoding enzymes with lignocellulolytic function were expressed under simulated N deposition (Kellner *et al.* 2010), we do not yet understand whether their overall expression levels (i.e., copy number/g) have been diminished by simulated N deposition. Collectively, our observations are consistent with the idea that chronic N deposition can: (i) repress lignolytic activity by the forest floor saprotrophic community; (ii) modify ecological interactions among these organisms, which subsequently alters community composition; and (iii) slow the long-term rate of litter decay, while increasing dissolved organic C production, and fostering organic matter accumulation.

Conclusions

Determining whether compositional shifts elicit functional responses by soil microbial communities lies at the heart of understanding ecological interactions in soil, as well as anticipating how environmental change will alter microbial

communities and the ecosystem processes they mediate. However, developing this understanding remains an elusive goal for reasons that are more practical than conceptual. For example, biogeochemical responses to environmental change can occur on decadal time steps in soil, but ecological interactions among saprotrophic organisms in soil occur on time step of hours to days to months. Underlying both of these ecological processes are the expression of microbial genes that influence the competitive ability of soil saprotrophs to obtain energy from plant detritus, a molecular process that operates at times steps of seconds to minutes. How we integrate molecular-level information to understand competitive interactions among soil saprotrophs, as well as how we further integrate across time to understand biogeochemical responses, is a challenging task. We do not yet understand how to link gene expression to the activity of extracellular enzymes which harvest energy for saprotrophic growth, nor do we understand how the activity of particular extracellular enzymes influences competitive interactions and the long-term response of biogeochemical processes. This knowledge gap extends to most facets of soil microbial ecology, and it remains an important scientific challenge. Our observations support the idea that molecular-level responses, such as enzyme activities, are consistent with the decadal ecosystem response of soil C cycling to simulated N deposition. We suggest that compositional shifts in soil microbial communities, mediated by ecological interactions among soil saprotrophs, lie at the biogeochemical heart of ecosystem response to environmental change.

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